

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Caselli RJ, Dueck AC, Osborne D, et al. Longitudinal modeling of age-related memory decline and the *APOE*  $\epsilon$ 4 effect. *N Engl J Med* 2009;361:255-63.

## ONLINE SUPPLEMENTARY APPENDIX

### **Methods: Longitudinal Growth Modeling**

To isolate the longitudinal effect of age on cognitive measures in this cross-sectional and longitudinal data set, we used a statistical method which simultaneously models the cross-sectional and longitudinal effect of age on each cognitive measure (30). We focus discussion on the longitudinal effect because the cross-sectional effect is potentially masked by selection bias (i.e., subjects were required to be cognitively normal at baseline limiting the cross-sectional effect of age on each cognitive measure). The acceleration of the rate of decline for each of the predetermined measures for carriers (collectively and also separately for each of the subgroups of homozygous and heterozygous persons) was compared to those of the non-carriers using a mixed model approach for modeling cross-sectional and longitudinal data (30,31). The model for  $Y_{ij}$  (the  $j^{\text{th}}$  response for the  $i^{\text{th}}$  individual) is as follows:

$$\begin{aligned} E(Y_{ij}|b_{1i}) = & \beta_1 + \beta_2 \text{Carrier}_i + \beta_3 \text{Agec}_{i1} + \beta_4 \text{Carrier}_i \times \text{Agec}_{i1} + \beta_5 \text{Agec}_{i1}^2 \\ & + \beta_6 \text{Carrier}_i \times \text{Agec}_{i1}^2 + \beta_7 \text{Agec}_{ij} + \beta_8 \text{Carrier}_i \times \text{Agec}_{ij} + \beta_9 \text{Agec}_{ij}^2 \\ & + \beta_{10} \text{Carrier}_i \times \text{Agec}_{ij}^2 + b_{1i} , \end{aligned}$$

where  $\text{Carrier}_i$  is the carrier status for the  $i^{\text{th}}$  individual (1=Carrier; 0=Non-Carrier);  $\text{Agec}_{ij}$  is the age minus 60 (i.e., centered age) of the  $i^{\text{th}}$  individual at the time of the  $j^{\text{th}}$  response; and  $b_{1i}$  is an individual specific random effect allowing each subject to have a different intercept. From this, the longitudinal model for non-carriers is given by:

$$E(Y_{ij}-Y_{i1}) = \beta_7 (Agec_{ij} - Agec_{i1}) + \beta_9 (Agec_{ij}^2 - Agec_{i1}^2),$$

and the longitudinal model for carriers is given by:

$$E(Y_{ij}-Y_{i1}) = (\beta_7 + \beta_8)(Agec_{ij} - Agec_{i1}) + (\beta_9 + \beta_{10})(Agec_{ij}^2 - Agec_{i1}^2).$$

A quadratic model was selected to allow for comparison of the acceleration in the rate of decline between groups. Age is centered to reduce the correlation between the age and age-squared terms and to aid in the interpretation of coefficients (e.g.,  $\beta_1$  represents the mean response for a 60-year-old non-carrier). From these models, a test of significance of  $\beta_{10}$  was used to assess the difference (between carriers and non-carriers) in the quadratic longitudinal effect of aging on the outcome measure being modeled. Modeling was carried out using SAS PROC MIXED (SAS Version 9).

In subsequent analyses, the model was modified to replace the  $Carrier_i$  variable (1) with a continuous variable equal to 0 for non-carriers, 1 for heterozygous persons, and 2 for homozygous persons used only to test for linear trend associated with allele-dose; and (2) with two indicator variables to assess differences between non-carriers and heterozygous persons ( $Hetero_i$ : 1=Heterozygous person; 0=Other) and between non-carriers and homozygous persons ( $Homo_i$ : 1=Homozygous person; 0=Other) used for all other reported results. These analyses were prespecified but considered exploratory given the small number of homozygous participants. Baseline characteristics and those recorded during follow-up were compared among groups by using the two-sample t-test / analysis of variance (ANOVA) F-test or Pearson chi-square test.

**Table S1.** Mixed Models of APOE e4 Carriers and Non-carriers

	AVLT			MMSE			COWAT			JLO		
	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
<b>Fixed Effects</b>												
<i>Intercept</i>	9.19	0.16	< <b>0.001</b>	29.52	0.05	< <b>0.001</b>	44.57	0.60	< <b>0.001</b>	24.79	0.20	< <b>0.001</b>
<i>Carrier<sub>i</sub></i>	-0.34	0.26	0.20	0.02	0.09	0.86	-0.26	0.97	0.79	0.29	0.33	0.37
<i>Age<sub>i1</sub>-60</i>	-0.0853	0.0254	< <b>0.001</b>	-0.0234	0.0102	<b>0.02</b>	-0.3979	0.0809	< <b>0.001</b>	-0.1118	0.0342	<b>0.001</b>
<i>Carrier<sub>i</sub> x (Age<sub>i1</sub>-60)</i>	0.0381	0.0379	0.31	0.0277	0.0150	0.07	-0.0189	0.1213	0.88	0.1360	0.0510	<b>0.008</b>
<i>(Age<sub>i1</sub>-60)<sup>2</sup></i>	0.0021	0.0013	0.11	0.0006	0.0005	0.27	0.0078	0.0041	0.06	0.0017	0.0016	0.28
<i>Carrier<sub>i</sub> x (Age<sub>i1</sub>-60)<sup>2</sup></i>	0.0031	0.0020	0.13	0.0000	0.0008	0.96	0.0035	0.0065	0.59	0.0000	0.0025	>0.99
<i>Age<sub>ij</sub>-60</i>	0.0408	0.0244	0.10	0.0060	0.0101	0.55	0.3406	0.0743	< <b>0.001</b>	0.0692	0.0331	<b>0.04</b>
<i>Carrier<sub>i</sub> x (Age<sub>ij</sub>-60)</i>	-0.0685	0.0351	0.05	-0.0309	0.0145	<b>0.03</b>	-0.0233	0.1065	0.83	-0.1257	0.0479	<b>0.009</b>
<i>(Age<sub>ij</sub>-60)<sup>2</sup></i>	-0.0013	0.0012	0.28	-0.0008	0.0005	0.09	-0.0116	0.0035	<b>0.001</b>	-0.0020	0.0015	0.18
<i>Carrier<sub>i</sub> x (Age<sub>ij</sub>-60)<sup>2</sup></i>	-0.0041	0.0018	<b>0.03</b>	-0.0002	0.0007	0.75	-0.0031	0.0055	0.57	-0.0006	0.0022	0.78
<b>Random Effects</b>												
<i>Intercept</i>	6.55	0.41	< <b>0.001</b>	0.60	0.06	< <b>0.001</b>	102.40	6.01	< <b>0.001</b>	9.42	0.62	< <b>0.001</b>
<i>Residual</i>	3.61	0.13	< <b>0.001</b>	0.64	0.03	< <b>0.001</b>	32.42	1.19	< <b>0.001</b>	4.85	0.20	< <b>0.001</b>

AVLT = Auditory Verbal Learning Test (Long Term Memory Score); MMSE = Mini Mental Status Examination; COWAT =

Controlled Oral Word Association Test; JLO = Judgment of Line Orientation; SE = Standard Error

**Table S2.** Mixed Models of APOE e4 Homozygous Persons, Heterozygous Persons, and Non-carriers

	AVLT			MMSE			COWAT			JLO		
	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
<b>Fixed Effects</b>												
<i>Intercept</i>	9.19	0.16	< <b>0.001</b>	29.52	0.05	< <b>0.001</b>	44.57	0.60	< <b>0.001</b>	24.79	0.20	< <b>0.001</b>
<i>Hetero<sub>i</sub></i>	-0.32	0.29	0.27	0.05	0.10	0.61	-1.34	1.09	0.22	0.37	0.36	0.31
<i>Homo<sub>i</sub></i>	-0.42	0.42	0.32	-0.08	0.14	0.59	2.74	1.57	0.08	0.12	0.54	0.83
<i>Age<sub>il</sub>-60</i>	-0.0853	0.0254	< <b>0.001</b>	-0.0234	0.0102	<b>0.02</b>	-0.3979	0.0809	< <b>0.001</b>	-0.1117	0.0342	<b>0.001</b>
<i>Hetero<sub>i</sub> x (Age<sub>il</sub>-60)</i>	0.0020	0.0420	0.96	0.0274	0.0167	0.10	-0.0611	0.1337	0.65	0.1248	0.0564	<b>0.03</b>
<i>Homo<sub>i</sub> x (Age<sub>il</sub>-60)</i>	0.0575	0.0658	0.38	0.0241	0.0247	0.33	0.0879	0.2243	0.70	0.1548	0.0870	0.08
<i>(Age<sub>il</sub>-60)<sup>2</sup></i>	0.0021	0.0013	0.11	0.0006	0.0005	0.27	0.0078	0.0041	0.06	0.0017	0.0016	0.28
<i>Hetero<sub>i</sub> x (Age<sub>il</sub>-60)<sup>2</sup></i>	0.0017	0.0022	0.44	-0.0010	0.0009	0.26	0.0033	0.0070	0.64	-0.0021	0.0027	0.44
<i>Homo<sub>i</sub> x (Age<sub>il</sub>-60)<sup>2</sup></i>	0.0052	0.0042	0.22	0.0032	0.0016	<b>0.049</b>	0.0007	0.0141	0.96	0.0065	0.0052	0.21
<i>Age<sub>ij</sub>-60</i>	0.0408	0.0244	0.09	0.0060	0.0101	0.55	0.3407	0.0743	< <b>0.001</b>	0.0691	0.0331	<b>0.04</b>
<i>Hetero<sub>i</sub> x (Age<sub>ij</sub>-60)</i>	-0.0279	0.0394	0.48	-0.0315	0.0163	0.05	0.0138	0.1196	0.91	-0.1200	0.0532	<b>0.02</b>
<i>Homo<sub>i</sub> x (Age<sub>ij</sub>-60)</i>	-0.1457	0.0497	<b>0.003</b>	-0.0273	0.0206	0.18	-0.0930	0.1509	0.54	-0.1257	0.0702	0.07
<i>(Age<sub>ij</sub>-60)<sup>2</sup></i>	-0.0013	0.0012	0.28	-0.0008	0.0005	0.08	-0.0116	0.0035	<b>0.001</b>	-0.0020	0.0015	0.18
<i>Hetero<sub>i</sub> x (Age<sub>ij</sub>-60)<sup>2</sup></i>	-0.0027	0.0020	0.18	0.0007	0.0008	0.36	-0.0006	0.0060	0.93	0.0014	0.0024	0.57
<i>Homo<sub>i</sub> x (Age<sub>ij</sub>-60)<sup>2</sup></i>	-0.0083	0.0032	<b>0.009</b>	-0.0034	0.0013	<b>0.01</b>	-0.0123	0.0096	0.20	-0.0080	0.0039	<b>0.04</b>
<b>Random Effects</b>												
<i>Intercept</i>	6.53	0.41	< <b>0.001</b>	0.60	0.06	< <b>0.001</b>	101.97	6.00	< <b>0.001</b>	9.46	0.62	< <b>0.001</b>
<i>Residual</i>	3.59	0.13	< <b>0.001</b>	0.64	0.03	< <b>0.001</b>	32.43	1.19	< <b>0.001</b>	4.84	0.20	< <b>0.001</b>

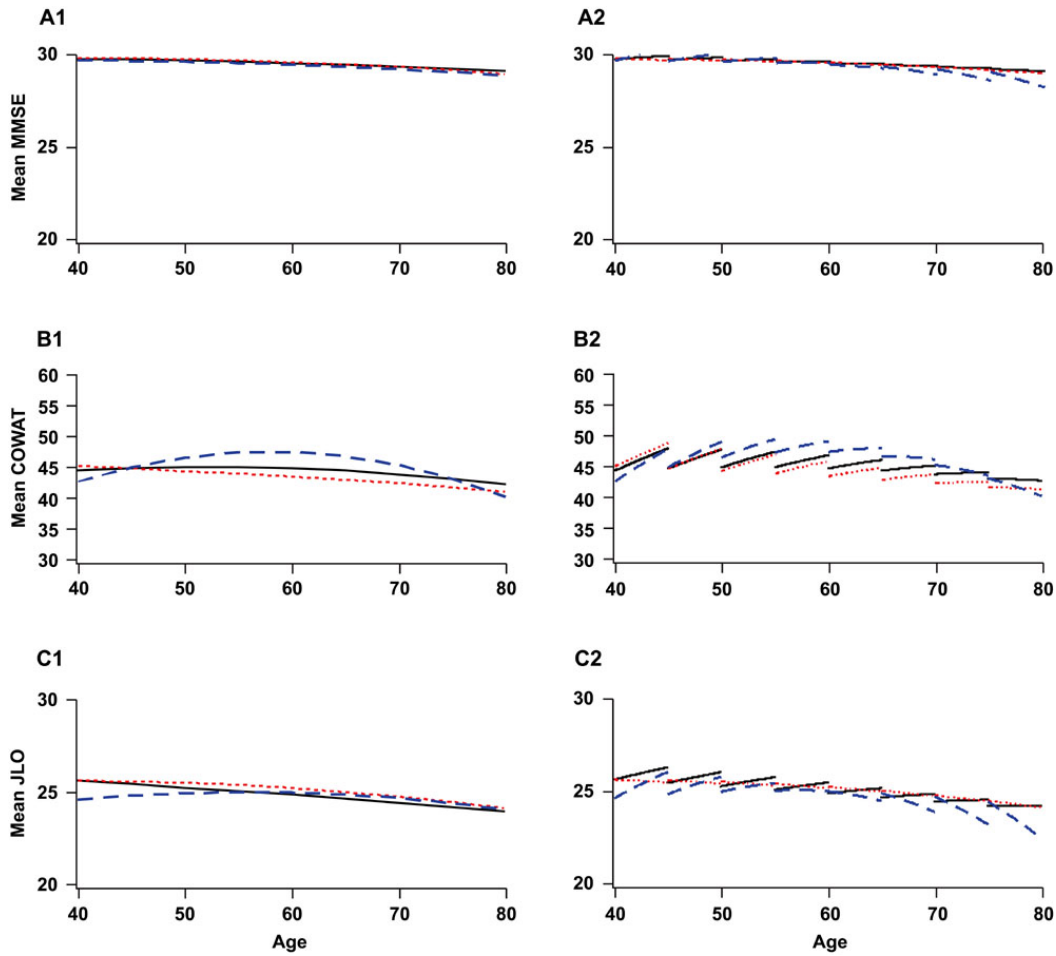
AVLT = Auditory Verbal Learning Test (Long Term Memory score); MMSE = Mini Mental Status Examination; COWAT = Controlled Oral Word Association Test; JLO = Judgment of Line Orientation; SE = Standard Error

**Table S3.** Observed and Fitted Mean Auditory Verbal Learning Test Long Term Memory Score at First Examination and Annual Change by Age Deciles: APOE e4 Homozygous Persons, Heterozygous Persons, and Non-carriers

AVLT-LTM at First Examination												
	Non-carriers				Heterozygotes				Homozygotes			
	Observed <sup>1</sup>			Model <sup>2</sup>	Observed <sup>1</sup>			Model <sup>2</sup>	Observed <sup>1</sup>			Model <sup>2</sup>
Age Decile	N	Mean	SD		N	Mean	SD		N	Mean	SD	
20-29	9	12.44	2.07	12.26	6	11.00	2.97	11.03	0	.	.	.
30-39	22	10.64	2.63	10.27	19	9.84	2.59	10.07	5	11.20	3.83	10.77
40-49	27	10.48	2.42	10.44	32	10.25	3.47	9.93	4	8.00	4.24	8.80
50-59	169	9.63	3.10	9.50	71	9.77	2.99	9.53	43	10.16	3.30	9.62
60-69	148	8.89	2.91	8.84	58	8.31	3.52	8.32	20	7.50	3.50	7.72
70-79	83	8.70	3.53	8.73	33	8.36	2.68	8.00	7	7.29	3.25	7.55
80-89	29	9.38	2.97	8.81	14	6.43	3.52	6.44	0	.	.	.
90-99	4	10.50	4.43	9.18	1	11.00	.	10.25	0	.	.	.
Annual Change in AVLT-LTM												
	Non-carriers				Heterozygotes				Homozygotes			
	Observed <sup>3</sup>			Model <sup>4</sup>	Observed <sup>3</sup>			Model <sup>4</sup>	Observed <sup>3</sup>			Model <sup>4</sup>
Age Decile	N	Mean	SD		N	Mean	SD		N	Mean	SD	
20-29	1	0.25	.	0.14	2	0.50	0.71	0.33	2	0.50	0.71	0.66
30-39	12	-0.50	1.06	0.12	9	0.33	0.59	0.25	3	0.26	0.41	0.47
40-49	18	0.12	0.82	0.09	13	0.14	1.28	0.17	3	0.11	0.34	0.28
50-59	92	0.10	0.81	0.07	49	-0.03	1.05	0.09	27	-0.03	0.77	0.09
60-69	134	-0.05	1.18	0.04	57	-0.04	1.23	0.01	31	0.13	1.08	-0.10
70-79	78	0.12	0.99	0.02	29	-0.80	1.83	-0.07	10	-0.05	0.82	-0.30
80-89	39	-0.33	1.66	-0.01	13	-0.25	1.33	-0.14	1	-3.15	.	-0.49
90-99	8	0.21	1.39	-0.03	1	0.48	.	-0.22	1	0.48	.	-0.68

AVLT = Auditory Verbal Learning Test (Long Term Memory score).

(1) Observed AVLT score at first examination per subject summarized for each age decile. (2) Predicted (empirical best linear unbiased predictor) AVLT score at first examination per subject based on the AVLT mixed model (see Online Supplement Table S2) averaged for each age decile. (3) Slope of simple linear regression line computed per subject (using all observations for that subject within the age decile) summarized for each age decile. (4) Predicted annual change in AVLT at the beginning of the age decile based on the AVLT mixed model (see Online Supplement Table S2).



**Figure S1.** Mean cross-sectional (1) and 5-year longitudinal trajectories (2) of the MMSE (A), COWAT (B), and JLO (C) by ApoE e4 carrier status (non-carrier vs heterozygous person vs homozygous person) based on the mixed model (Online Supplemental Table S2). Cross-sectional means represent population mean values at the first examination. Longitudinal mean trajectories are population mean trajectories for ages (at first examination) at 5-year increments. Predicted values at the first examination and predicted trajectories for each subject in the sample (not shown) are vertically shifted from the displayed means via the random intercept term. APOE e4 non-carriers (solid black line), heterozygous persons (dotted red line), and homozygous persons (dashed blue line).